Claims

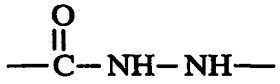
1. A method of producing an oligopeptide product, the method comprising the steps:

a) providing a first oligopeptide, the first oligopeptide having a reactive moiety,

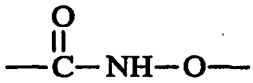
b) providing a second oligopeptide, the second oligopeptide having a activated ester moiety

c) allowing the reactive moiety of the first oligopeptide to react with the activated ester moiety of the second oligopeptide to form an oligopeptide product, in which the first and second oligopeptides are linked via a linking moiety having Formula I, Formula II or Formula III.

Formula I



Formula II



Formula III

2. The method according to claim 1 wherein the terminal activated ester moiety is a thioester wherein the peptide is the acyl substituent of

the thioester.

3.The method according to claim 2, wherein said second polypeptide is generated by thiol reagent dependent cleavage of a precursor molecule, said precursor molecule comprising a second oligopeptide fused N-terminally to an intein domain.

4. A method of producing an oligopeptide product, the method comprising the steps:
a) providing a first oligopeptide, the first oligopeptide having a reactive moiety,
(i) providing a precursor oligopeptide molecule, the precursor oligopeptide molecule comprising a second oligopeptide fused N-terminally to an intein domain
(ii) allowing thiol reagent dependent cleavage of the precursor molecule to generate a second oligopeptide molecule, said second oligopeptide molecule having a thioester moiety at its C-terminus,
c) allowing the reactive moiety of the first oligopeptide to react with the second oligopeptide molecule to form an oligopeptide product, in which the first and second oligopeptides are linked via a linking moiety having Formula I, II or III.

5. The method according to any one of the preceding claims wherein the reactive moiety is a hydrazine moiety, a hydrazide moiety or an aminooxy moiety.

6. The method according to claim 5, wherein the reactive moiety is an aminooxy moiety and the

activated ester moiety is a thioester.

7. The method according to claim 5, wherein said first oligopeptide is produced by reaction of hydrazine with a precursor molecule, said precursor molecule comprising a precursor oligopeptide fused N-terminally to an intein domain via a thioester moiety.

8. A method of producing an oligopeptide product, said method comprising the steps:
a) providing a first oligopeptide, the first oligopeptide having a reactive moiety, wherein the reactive moiety is a hydrazine moiety, a hydrazide moiety or an amino-oxy moiety;
(i) providing a precursor oligopeptide molecule, the precursor oligopeptide molecule comprising a second oligopeptide fused N-terminally to an intein domain;
(c) allowing the reactive moiety of the first oligopeptide to react with the precursor oligopeptide molecule to form an oligopeptide product, in which the first and second oligopeptides are linked via a linking moiety having Formula I, Formula II or Formula III.

9. The method according to any one of the preceding claims, wherein the first oligopeptide or the second oligopeptide is a recombinant oligopeptide and the other of the the first oligopeptide and the second oligopeptide is a synthetic polypeptide.

10. The method according to any one of claims 1 to 8, wherein the first oligopeptide and the second oligopeptide are recombinant oligopeptides.

11. The method according to any one of claims 1 to 8, wherein the first oligopeptide and the second oligopeptide are synthetic oligopeptides.

12. A method of generating a protein hydrazide, said method comprising the steps:
(a) providing a protein molecule comprising an oligopeptide fused N-terminal to an intein domain,
(b) reacting said protein molecule with hydrazine, such that the intein domain is cleaved from the oligopeptide to generate a protein hydrazide.

13. The method according to any one of the claims 1 to 11 wherein step (c) of the method is performed at a pH in the range pH 6.5 to 7.5.

14. A method of producing an oligopeptide product, the method comprising the steps:
a) providing a first oligopeptide, the first oligopeptide having an aldehyde or ketone moiety,
b) providing a precursor oligopeptide molecule, the precursor oligopeptide molecule comprising a second oligopeptide fused N-terminally to an intein domain,
c) reacting said precursor oligopeptide molecule

with hydrazine to generate an oligopeptide molecule comprising an intermediate oligopeptide, said intermediate oligopeptide having a terminal hydrazide moiety,

d) allowing the aldehyde or ketone moiety of the first oligopeptide to react with the hydrazide moiety of the intermediate oligopeptide molecule to form an oligopeptide product, in which first oligopeptide and the second oligopeptide are linked via a hydrazone linking moiety.

15. An oligopeptide product produced by the method of any one of the preceding claims.

16. A method of labelling an oligopeptide, the method comprising the steps:

a) providing a label molecule, the label molecule having a reactive moiety,

b) providing the oligopeptide, the oligopeptide having a activated ester moiety

c) allowing the reactive moiety of the label molecule to react with the activated ester moiety of the oligopeptide to form the labelled oligopeptide, in which the label molecule and the oligopeptide are linked via a linking moiety having Formula I, Formula II or Formula III.

17. The method according to claim 16, wherein in step (c), where said label molecule and the oligopeptide are linked via a linking moiety having Formula II and where said activated ester moiety of step (b) is not a thioester, said

activated ester is a terminal activated ester moiety.

18. A method of labelling an oligopeptide, the method comprising the steps:
a) providing a label molecule, the label molecule having an activated ester moiety of which the label is the acyl substituent,
b) providing the oligopeptide, the oligopeptide having a reactive moiety
c) allowing the activated ester moiety of the label molecule to react with the reactive moiety of the oligopeptide to form the labelled oligopeptide, in which the label molecule and the oligopeptide are linked via a linking moiety having Formula I, Formula II or Formula III, wherein, in step (c), where said label molecule and the oligopeptide are linked via a linking moiety having Formula II and where said activated ester moiety of step (b) is not a thioester, said activated ester is a terminal activated ester moiety.

19. The method according to claim 18 wherein said oligopeptide is produced by reaction of hydrazine with a precursor molecule, said precursor molecule comprising a precursor oligopeptide fused N-terminally to an intein domain via a thioester moiety.

20. A method of labelling an oligopeptide, the method comprising the steps:

a) providing a label, the label having a reactive moiety,
(i) providing a precursor oligopeptide molecule, the precursor oligopeptide molecule comprising an oligopeptide fused N-terminally to an intein domain
(ii) allowing thiol reagent dependent cleavage of the precursor molecule to generate the oligopeptide molecule, said oligopeptide molecule having a thioester moiety at its C-terminus,
c) allowing the reactive moiety of the label to react with the oligopeptide molecule to form a labelled oligopeptide, in which the label and oligopeptide are linked via a linking moiety having Formula I, II or III.

21. The method according to any one of claims 16 to 18, wherein the reactive moiety is an aminooxy moiety and the activated ester moiety is a thioester.

22. The method according to claim 20, wherein the reactive moiety is an aminooxy moiety.

23. A method of labelling an oligopeptide, the method comprising the steps:
a) providing a label molecule, the label molecule having a reactive moiety,
b) providing a precursor oligopeptide molecule, the precursor oligopeptide molecule comprising an oligopeptide fused N-terminally to an intein domain,

c) allowing the reactive moiety of the label molecule to react with the precursor oligopeptide molecule to form a labelled oligopeptide product, in which the label molecule and the oligopeptide are linked via a linking moiety having Formula I, Formula II or Formula III as defined above.

24. The method according to any one of claims 16 to 23 wherein step (c) of the method is performed at a pH in the range pH 6.5 to pH 7.5.

25. A method of labelling an oligopeptide, the method comprising the steps:
a) providing a label molecule, the label molecule having a aldehyde or ketone moiety,
b) providing a precursor oligopeptide molecule, the precursor oligopeptide molecule comprising a first oligopeptide fused N-terminally to an intein domain,
c) reacting said precursor oligopeptide molecule with hydrazine to generate an oligopeptide molecule comprising an intermediate oligopeptide, said intermediate oligopeptide having a terminal hydrazide moiety,
d) allowing the aldehyde or ketone moiety of the label molecule to react with the hydrazide moiety of the intermediate oligopeptide molecule to form a labelled oligopeptide product, in which the label molecule and oligopeptide are linked via a hydrazone linking moiety.

26. The method according to claim 14 or claim 25, wherein the aldehyde or ketone moiety is an α-diketone or an α-keto-aldehyde group.

27. A labelled oligopeptide produced by the method of any one of claims 16 to 26.